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Antagonism of Depressant Activity of Ethanol by DH–524; a Comparative Study with Bemegride, Doxapram and *d*-Amphetamine (38640)

ABDULMUNIEM H. ABDALLAH AND DOUGLAS M. ROBY
(Introduced by F. N. Marshall)

The Dow Chemical Company, Chemical Biology Research, Midland, Michigan 48640

Alcoholism is a major public health problem in the United States as well as in most of the Western world (Rubin and Lieber, 1971). Moreover, maternal alcoholism may cause developmental and physical defects in babies (Ulleland, 1970, Smith *et al.*, 1973). The acute administration of ethanol causes central nervous system depression and lowers the body temperature of mice (Freund, 1973). It was reported that DH-524 is more selective than the analeptics in antagonizing the effects of some central nervous system depressants (Reitz and others, 1973). The purpose of this study was to compare the effects of DH-524 (2(3,4-dichlorophenoxy)-methyl-2-imidazoline), bemegride, doxapram and d-amphetamine on the narcotic and hypothermic effect of ethanol in mice.

Materials and Methods. Male Swiss–Origin-ICR mice derived from Harlan Industries Cumberland, Indiana (of approximately the same age and weight) were used in all studies. Sleeping times are defined as the time from the intraperitoneal injection of alcohol until the animal was able to right itself twice. All experiments were done in a quiet room at constant temperature (25.0 ± 1.0°) between 8.0 AM and 2.0 PM.

Antagonism of ethanol narcosis. Mice were divided into groups at random. Each group was injected intravenously either with the test drugs or with saline and placed into a plastic observation cages. Five minutes later each mouse was injected with 4.4 ml/kg ip of ethanol (4.4 ml of 100% ethanol diluted with water to make 10 ml). The mice were then placed on their backs as soon as narcosis developed and the time of the first and second righting was recorded. The mean sleep time of drug pretreated groups was compared to that of saline pretreated groups. Results were analyzed by the Student's *t* test.

Antagonism of ethanol induced hypothermia in mice. Mice were randomly divided (10 mice per group) and placed individually into plastic observation cages. Thirty minutes later initial rectal temperature was recorded. Each group was injected intravenously either with the test drugs or with saline. Five minutes later ethanol (3.8 ml/kg, ip of 100% ethanol diluted with water up to 10 ml) or saline was injected. Rectal temperature was recorded 30, 60, 90 and 120 min after ethanol injection. The mean temperatures of the drug pretreated group were compared to the saline pretreated group. Results were analyzed by the Student's *t* test.

Reversal of ethanol induced hypothermia in mice. The method used was essentially the same as in the aforementioned test. The rectal temperature of mice was recorded immediately before and 60 min after ethanol administration. Test drugs or saline were injected iv 60 min after ethanol administration. The body temperature of mice was recorded at 30, 60, 90 and 120 min after drug administration. The mean body temperature of the drug treated group at each interval was compared to that of the saline treated group. Results were analyzed by the Student's *t* test.

Total spontaneous motor activity of mice (TSMA). Naive, male mice weighing 20–25 g were used in this study. Mice were randomly divided into groups of 10 each. Each group received a different dose of the drug tested. One group received saline and served as a control. Each mouse was injected (iv) either with drug or saline and was placed on top of the sensor (Stoelting Co.), and the TSMA recorded for 60 min. Each time the mouse interrupts the electromagnetic field it registers a count. Counts of drug treated groups were compared to saline treated groups according to Student's *t* test.

Bemegride was obtained as a commercial intravenous preparation (5 mg/ml Abbott

TABLE I. EFFECT OF DH-524, BEMEGRIDE, DOXAPRAM AND *d*-AMPHETAMINE PRETREATMENT ON ETHANOL (4.4 ml/kg, ip) SLEEP TIME IN MICE.

Experiment no.	Pretreatment	Dose (mg/kg iv)	N[c]	Mean sleep time (min.) + SE
1	Saline	—	10	52.7 ± 14.6
	DH-524	5	10	48.5 ± 12.1
	DH-524	10	10	36.2 ± 14.6
	DH-524	15	10	0.0 ± 0.0[e]
2	Saline	—	10	39.1 ± 15.1
	Bemegride[a]	10	10	39.9 ± 11.15
	Doxapram[a]	25	10	57.0 ± 10.8
	Doxapram[a]	50	10	97.4 ± 9.9[b]
3	Saline	—	10	35.4 ± 13.3
	d-Amphetamine	2	10	22.3 ± 4.6
4	Saline	—	10	43.4 ± 9.9
	d-Amphetamine	4	10	50.5 ± 11.6
	d-Amphetamine	8	10	30.4 ± 5.9
5	Saline	—	10	73.7 ± 12.6
	DH-524	11.5	10	34.9 ± 9.9[d]
	DH-524	13.5	10	15.4 ± 7.5[e]
	d-Amphetamine	25.0	10	17.0 ± 10.0[e]

[a] = These doses of bemegride and doxapram caused nonlethal convulsion in all mice tested.
[b] = Significantly more than saline treated group ($P < 0.01$).
[c] N = Number of mice per group.
[d] Significantly less than saline treated group ($P < 0.05$).
[e] Significantly less than saline treated group ($P < 0.01$).

Laboratories) as was doxapram (20 mg/ml A. H. Robins). *d*-Amphetamine and the 95% ethanol were obtained from Sigma Chem. Co. and U.S. Industrial Chemicals respectively. Dilutions of these preparations were made with saline. DH-524, Lot number L-1429 is a Dow Chemical Company compound.

Results. Table I shows that the administration of 11.5, 13.5 and 15 mg/kg doses of DH-524 and a 25 mg/kg dose of *d*-amphetamine caused significant antagonism of ethanol induced narcosis. On the other hand, bemegride and doxapram failed to have a significant effect on ethanol induced narcosis. In the case of doxapram, the 50 mg/kg dose caused a significant increase in sleep time.

The intravenous administration of 10 and 15 mg/kg of DH-524 significantly antagonized ethanol induced hypothermia in mice at 60, 90 and 120 min postdrug (Fig. 1). In the case of the group which received only ethanol (3.8 ml/kg) body temperatures were significantly reduced at all intervals. Figure 2 shows that bemegride and doxapram at the doses used failed to antagonize significantly ethanol-induced hypothermia. The 25 mg/kg dose of doxapram tended to potentiate ethanol hypothermia. In the case of amphetamine, the 2 mg/kg dose significantly antagonized ethanol induced hypothermia at 30, 60 and 90 min. Table II shows that both DH-524 and *d*-amphetamine reversed ethanol induced hypothermia. On the other hand, bemegride and doxapram appeared to be ineffective. All doses of bemegride and doxapram caused convulsions in mice. Therefore we did not study the effect of higher doses of these two compounds.

Discussion. Our results agree with those of Freund (1973) who reported that ethanol lowers body temperature of mice.

DH-524 significantly antagonized ethanol

TABLE II. REVERSAL OF ETHANOL (3.8 ml/kg,/ip) INDUCED HYPOTHERMIA BY THE ADMINISTRATION OF DH-524, BEMEGRIDE, DOXAPRAM AND *d*-AMPHETAMINE.

Treatment ml/kg (ip)	Posttreatment	Dose mg/kg (iv)	N[a]	Body temperature Mean + SE				
				Post-Ethanol at 60 min.	Post-Antagonists at 30	60	90	120 min
Saline	Saline	—	10	38.0 ± 0.13	37.6 ± 9.14	37.7 ± 0.10	37.8 ± 0.10	37.6 ± 0.12
Ethanol	Saline	—	10	35.3 ± 0.21[b]	34.7 ± 0.31[b]	34.7 ± 0.34[b]	34.9 ± 0.40[b]	35.0 ± 0.46[b]
Ethanol	DH-524	10	10	35.1 ± 0.20	35.0 ± 0.25	35.5 ± 0.31	36.0 ± 0.31[c]	36.1 ± 0.37
Ethanol	DH-524	15	10	34.9 ± 0.22	35.2 ± 0.21	35.7 ± 0.23[c]	36.5 ± 0.24[d]	36.6 ± 0.26[c]
Ethanol	Saline	—	10	33.7 ± 0.23	33.7 ± 0.19	33.8 ± 0.28	33.9 ± 0.30	33.9 ± 0.28
Ethanol	Bemegride	10	10	33.3 ± 0.21	34.2 ± 0.20	34.3 ± 0.17	34.4 ± 0.20	34.5 ± 0.23
Ethanol	Doxapram	25	10	33.6 ± 0.36	33.5 ± 0.31	33.6 ± 0.31	33.6 ± 0.32	33.8 ± 0.31
Ethanol	d-Amphetamine	2	10	33.9 ± 0.26	34.5 ± 0.26[d]	34.7 ± 0.29[d]	34.2 ± 0.28	34.2 ± 0.28

[a] N = Number of mice per group.
[b] = Significantly different from saline treated group ($P < 0.01$).
[c] = Significantly different from ethanol treated group ($P < 0.05$).
[d] = Significantly different from ethanol treated group ($P < 0.01$).

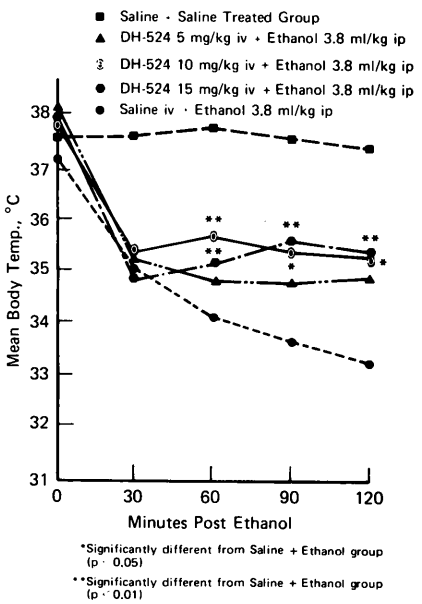

FIG. 1. Effect of DH-524 pretreatment on ethanol induced hypothermia in mice. Abscissa: Time in minutes. Ordinate: Mean body temperature. Closed Square: Saline plus saline treated group. Closed triangle: DH-524 mg/kg iv plus ethanol 3.8 ml/kg ip. Dotted circle: DH-524 10 mg/kg iv plus ethanol 3.8 ml/kg ip. Closed hexagonal: DH-524 15 mg/kg iv plus ethanol 3.8 ml/kg ip. Closed circle: Saline iv plus ethanol 3.8 ml/kg ip.

induced narcosis and hypothermia. On the other hand *d*-amphetamine antagonized only the hypothermic effect of ethanol in doses which caused a significant increase in SMA. However, when a high dose of *d*-amphetamine (25 mg/kg iv) was administered it caused a significant antagonism of ethanol narcosis. Moreover, unlike *d*-amphetamine, DH-524 does not significantly increase spontaneous motor activity of mice. (Table III).

Kimura and Richards (1957) reported that 10 mg/kg of bemegride antagonized pentobarbital induced narcosis. In our hands, all doses of bemegride and doxapram tested failed to antagonize the effects of ethanol in spite of the fact that they caused convulsions in mice. In fact, doxapram potentiated rather than antagonized, the effects of ethanol in proportion to dose. In conclusion, DH-524 differs from all other agents tested in that it antagonized the pharmacological effects of ethanol in doses which produced no other pharmacological effects.

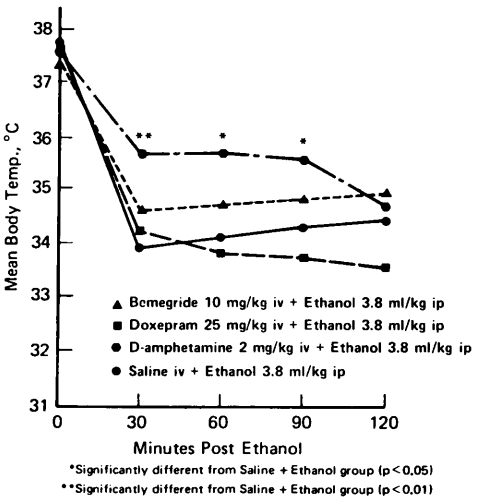

FIG. 2. Effect of bemegride, doxapram, *d*-amphetamine pretreatment on ethanol induced hypothermia in mice. Abscissa: Time in minutes post ethanol. Ordinate: Mean body temperature. Closed triangle: Bemegride 10 mg/kg iv plus ethanol 3.8 ml/kg ip. Closed square: Doxapram 25 mg/kg iv plus ethanol 3.8 ml/kg ip. Closed hexagonal: *d*-amphetamine 2 mg/kg iv plus ethanol 3.8 ml/kg ip. Closed circle: Saline iv plus ethanol 3.8 ml/kg ip.

TABLE III. EFFECT OF DH-524 AND *d*-AMPHETAMINE ON SPONTANEOUS MOTOR ACTIVITY OF MALE MICE.

Treatment	Dose (mg/kg iv)	N[a]	Mean Count ± SE in 60 min
Saline	—	10	827 ± 123
DH-524	15	10	1375 ± 671
d-Amphetamine	4	10	5243 ± 498[b]

[a] N = Number of mice per group.
[b] Significantly more than saline treated group ($P < 0.01$).

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